

Exhibit A

372.6435P

antisense reagents which bind to a domain of the open reading frame of P2P cDNA can be used to repress P2P expression and cellular proliferation, which indicates that the repression of P2P expression may be able to repress the proliferative potential of normal, nontransformed cells, abnormal cells, and cancerous cells both in vitro and in vivo. The results of these studies establish the therapeutic value of P2P antisense reagents for the treatment of proliferative diseases, including cancer.

The ATCC Accession Number for monoclonal antibody C130

is:

The following References are incorporated herein by reference:

1. Scott, R.E., Hoerl, B.J., Wille, J.J., Jr., Florine, D.L., Krawisz, B.R. and Hun, K. (1982) J. Cell Biol. 94, 400-405.
2. Tontoz, P., Erding, H. and Spiegelman, B.M. (1994) Cell 79, 1147-1156.
3. Smyth, M.J., Sparks, R.L. and Wharton, W. (1993) J. Cell Sci. 106, 1-9.
4. Smas, C.M. and Sul, H.S. (1995) Biochem. J. 309, 697-710.
5. McKnight, S.L. (1992) in Transcriptional Regulation, eds. McKnight, S.L. and Yamamoto, K.R. (Cold Spring Harbor Laboratory Press, Plainview, NY), pp.771-795.
6. Wier, M.L. and Scott, R.E. (1986) Am. J. Pathol. 125, 546-554.
7. Minoo, P., Sullivan, W., Solomon, L.R., Martin, T.E., Toft, D.O. and Scott, R.E. (1989) J. Cell Biol. 109, 1937-1946.
8. Scott, R.E. and Witte, M.M. (1993) Mol. & Cell. Diff. 1, 185-195.

placed proximal to the bacteriophage T7 gene 10 translation initiation site such that individual plasmids were isolated containing the cDNA in all six reading frames in phase with the gene 10 protein product. Expression of the protein encoded in each reading frame was obtained by infecting *E. coli* strain HMS174 harboring the recombinant plasmid with the bacteriophage CE6 as described above. This bacteriophage is a lambda-derived phage containing the gene for T7 RNA polymerase. Infected bacteria containing the recombinant pET5 vectors produce the T7 RNA polymerase which in turn directs the expression of fusion proteins between the T7 gene 10 protein and the reading frame of the cDNAs. Only one reading frame which corresponds to the 3' end of the large open reading frame [FIG. 1], resulted in expression of fusion proteins antigenically related to P2Ps. The fusion protein was electroeluted from preparative gels and used to produce a P2P-specific monoclonal antibody at the University of Tennessee, Memphis, Molecular Resource Center Hybridoma Laboratory.

One hybridoma so generated was reactive against the purified fusion protein. The antibody, termed C130, was therefore used to probe 3T3T nuclear and total cell extracts by Western analysis. FIG. 4 shows that the C130 monoclonal antibody specifically detects native P2P proteins in a manner similar to the pattern seen with AC88. However, C130 and AC88 recognize separate epitopes because C130 detects only P2Ps whereas AC88 shows cross-reactivity to heat shock protein 90. These data support the conclusion that the cloned P2P cDNA encode hnRNP-related P2P peptides. Evidence that the P2P cDNA Encodes a Rb1 Binding Peptide Using A P2P-GST Fusion Protein

Because Rb1 is required for muscle cell terminal differentiation (10) and data showing that P2P expression is modulated during terminal adipocyte differentiation state, studies were performed to determine if P2P cDNA products might interact with Rb1. To accomplish this GST-P2P fusion proteins were periodically produced to different P2P cDNA domains. Cellular lysates were prepared from human K-562 hematopoietic stem cells which contain abundant Rb1 protein and these lysates were then precipitated with each of the four GST-P2P fusion proteins, i.e. GST-P2P (1-332), (484-688), (753-908) and (918-1095) as illustrated in FIG. 1. The lysates were also precipitated with GST protein alone as a negative control in these experiments. FIG. 5A demonstrates that one fusion protein, GST-P2P (753-909), specifically precipitates a protein that is detected by the anti-Rb1 antibody IF8. FIG. 5a also shows that the GST-P2P (753-909) fusion protein preferentially binds the hypophosphorylated form of Rb-1 which is primarily expressed in the G₁ phase of the cell cycle thus suggesting a possible physiological role for the interaction of P2P cDNA products and Rb1 in the control of cell growth.

Most proteins that associate with the hypophosphorylated form of Rb1 bind to a region of Rb1 that has been termed the "pocket" domain (28). To determine if the interaction between Rb1 and GST-P2P (753-909) occurs through the Rb1 "pocket" domain, competition experiments were conducted using purified viral E1a protein. E1a is known to bind specifically to the Rb1 pocket domain and to inhibit cellular proteins from binding to this region (29). FIG. 5B shows that the interaction between the GST-P2P(753-909) fusion protein and Rb1 is blocked by the addition of purified E1a protein. This inhibition is specific for the E1a protein since the addition of another protein, dihydrofolate reductase, did not block the interaction of Rb1 and the GST-P2P fusion protein (data not shown). Therefore, GST-P2P(753-909) binds specifically to the hypophosphorylated form of Rb1 and this interaction occurs through the Rb1 "pocket" domain.

Gene Therapy Using P2P cDNA-Derived Antisense Oligonucleotide Reagents

P2P mRNA and P2P protein is expressed in cells that have proliferative potential regardless of whether they are in a growing or quiescent state. Conversely, the expression of P2P cDNA products is repressed in cells that have lost their proliferative potential as a result of terminal differentiation or senescence. In contrast, transformed cells with malignant characteristics, especially SV40 transformed cells that lack the ability to terminally differentiate or senesce, lack the ability to repress P2P expression. It is conceived, therefore, that the proliferative potential of cancer cells, in general, may be blocked if P2P expression is repressed by the use of antisense oligonucleotide reagents that are targeted to bind to specific domains of the P2P mRNA to block its translation.

The P2P antisense oligonucleotide [5' CAGCAGGAGCTGTGTT '3 cDNA (3424-3409)] shown by SEQ ID NO:3 and a P2P sense oligonucleotide [5' CTACTAAGC-CATCGGC '3 (3560-3575)] shown by SEQ ID NO:4 have been prepared, isolated, and studied, as shown below in Table I. The antisense oligonucleotides are prepared by Jude Labs (Memphis, Tenn.) and BioSynthesis (Louisville, Tex.). These oligonucleotides (15-50 mg/ml) were added to the culture media of growing 3T3T cells for various times up to 9 days and the effect of these treatments on P2P expression was determined by Western blotting using the AC88 antibody to detect P2Ps.

TABLE I

	Selective Repression of P2P Expression With Antisense Oligonucleotides	
	Repression of P2P Expression	Repression of Control Protein hsp90
P2P Antisense	83%	0%
P2P Sense	6%	0%

Additional data also suggests that a P2P antisense oligonucleotide can repress cellular proliferation by greater than 50%, whereas a P2P sense oligonucleotide has no effect. Thus, P2P antisense reagents which bind to a domain of the open reading frame of P2P cDNA can be used to repress P2P expression and cellular proliferation, which indicates that the repression of P2P expression may be able to repress the proliferative potential of normal, nontransformed cells, abnormal cells, and cancerous cells both in vitro and in vivo. The results of these studies establish the therapeutic value of P2P antisense reagents for the treatment of proliferative diseases, including cancer.

The monoclonal antibody C130 is commercially available from Santa Cruz Biotechnology under the designation PACT (M56).

THE FOLLOWING REFERENCES ARE INCORPORATED HEREIN BY REFERENCE

1. Scott, R. E., Hoerl, B. J., Wille, J. J., Jr., Florine, D. L., Krawisz, B. R. and Hun, K. (1982) *J. Cell Biol.* 94, 400-405.
2. Tontoz, P., Erding, H. and Spiegelman, B. M. (1994) *Cell* 79, 1147-1156.
3. Smyth, M. J., Sparks, R. L. and Wharton, W. (1993) *J. Cell Sci.* 106, 1-9.
4. Smas, C. M. and Sul, H. S. (1995) *Biochem. J.* 309, 697-710.
5. McKnight, S. I. (1992) in *Transcriptional Regulation*, eds. McKnight, S. L. and Yamamoto, K. R. (Cold Spring Harbor Laboratory Press, Plainview, N.Y.), pp. 771-795.

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CGAAGAGACG AGAGAGGTGA ATTAGCAAGG AGAAAAGACT CTCCTCCCCG GGGCAAAGAG 4020
TCTCTGTCTG GGCAGAAAAG CAAGCTGAGG GAGGAGAGAG ATTTACCTAA AAAGGGGGCC 4080
GAGTCAAAAA AAGTAATTC TAGCCCCCA AGAGACAAAA AGCCTCATGA TCATAAGSCC 4140
CCCTACGAAA CTAAACGCCC ATGTGAAGAG ACAAGCCTG TAGATAAAAA CTCTGGGAAG 4200
GAGCGGGAGA AGCATGCTGC TGAAGCTCGC AATGGGAAAG AGTCCAGTGG TGCAAAGTGC 4260
CATGTATACC TAACCCGCCA GACCCCTCCA TGGAGAAGGA GCTGGCTGCT GGGCAGGTGG 4320
AGAAGAGCGC CGTCAAGCCG AAACCCAGC TGAGCCATTC CTCGAGGCTT TCCTCTGACC 4380
TGACCCGGGA GACGAACGAG GCAGCCTTTG AACGAGATTA TAATGAGAGC GACAGTGAGA 4440
GTAATGTGTC TGTGAAGGAA GAAGAAGCTG TTGCCAGTAT CTCCAAGGAC TTGAAAGAGA 4500
AAACAACAGA GAAAGCGAAA GAGAGCTTGA CTGTAGCAAC GGCCAGCCAG CCAGGTGCAG 4560
ACAGGAGCCA GAGCCAAAGT AGCCCACTGT TAGTCAGTAG AGTCATAGCC TTCGGAGCCA 4620
GACCCGAAGC CACAGCAGCA GTGCCAGCTC AGCCGGAAGG CCAGGACAGC AAAAAGAAGA 4680
AGAAGAAGAA GGAGAAGAAA AACGACAAGA AGCATAAAAA GCACAAGAAG CACAAGAAGC 4740
ACCGAGGCCG ACGGCGACGT GGAGAAGAGC CAGAAACACA AACACAAGAA GAAGAAGGCC 4800
AAGAAGAACA AAGACAAGGA GAAGGAGAAA GATGACCAAA AAGTGAGATC TGTCACTGTG 4860
TGAAGGACGG ATGTGTTAAT TGACTTAATT ACTAAGTCAT CTGTATTAAA TTCTGTTATA 4920
ATGTAAAGAG ATTCCAGCCT TGTAATAAT GAATGAAGA CCCTGTGCTG CACTTAAAG 4980
TATTTGCTGC TTGATTATTT CATTTTACA TCAGAGCTTT ATAACGAAT TTTGTACAGA 5040
ATTGTGAGTT GTGACCATGG AACAGTGAGA GGTTTTGCTA GGGCTATTA TTTTAAACCA 5100
CCATTAATTA GTTGGGTGG AGTTTACTGT ACTGTGAAAT TTTCACATTT GAATTTTTTT 5160
AATTGCCTGG CAA 5173

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CAGCAGGAGC TGTGTT

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(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CTACTAAGCC ATCGGC

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What is claimed is:

1. An isolated DNA molecule comprising SEQ ID NO:2.
2. The DNA sequence of claim 1 with its complementary strand.

3. The DNA molecule of claim 1 which hybridizes under stringent conditions to a transcript of approximately 8 kilobases in murine liver, kidney, testes, lung and in murine 3T3T stem cells.

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4. The DNA molecule of claim 1 which is not expressed in terminally differentiated 3T3T cells or in senescent human keratinocytes.

5. The DNA molecule of claim 1 which comprises an open reading frame of at least 4814 nucleotides.

6. The DNA molecule of claim 1 which encodes the P2P polypeptide.

7. The DNA molecule of claim 1 which is mouse DNA.

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8. An isolated ribonucleic acid molecule comprising a transcript encoding a P2P protein comprising the amino acid sequence of SEQ ID No. 1.

9. An isolated antisense oligonucleotide which anneals to a complementary portion of a mRNA transcript comprising SEQ ID No. 2, wherein binding of said antisense oligonucleotide to said mRNA transcript represses expression of P2P gene.

10. The antisense oligonucleotide of claim 9, wherein said antisense oligonucleotide anneals to an open reading frame of said mRNA transcript under physiological conditions.

11. The antisense oligonucleotide of claim 5, wherein said antisense oligonucleotide is complementary to an open reading frame of said mRNA transcript encoding a P2P protein having an amino acid sequence of SEQ ID No. 1.

12. The antisense oligonucleotide of claim 9 SEQ ID NO:3.

13. A method of inhibiting the expression of a gene encoding a P2P protein, comprising the step of:

contacting growing cells selected from the group consisting of normal, abnormal, and cancer cells with the antisense oligonucleotide of claim 9.

14. The method of claim 13 wherein the antisense oligonucleotide comprises SEQ ID NO:3.

15. A method for repressing the proliferative potential of a cell, comprising the step of:

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contacting growing cells selected from the group consisting of normal, abnormal, and cancer cells with the antisense oligonucleotide of claim 9, wherein inhibition of the expression of P2P gene by said antisense oligonucleotide represses the proliferative potential of said cell.

16. The method of claim 15 wherein the antisense oligonucleotide comprises SEQ ID NO:3.

17. A method for inhibiting the translation of a P2P mRNA transcript in cells, comprising the step of:

contacting cells selected from the group consisting of normal, abnormal, and cancer cells with the antisense oligonucleotide of claim 9, wherein said antisense oligonucleotide anneals to the portion of the P2P mRNA transcript encoding a C-terminal region of the P2P protein comprising the domain that binds to RNA.

18. The method of claim 17 wherein said antisense oligonucleotide anneals to the portion of the P2P mRNA encoding one or more of epitopes recognized by antibodies selected from the group consisting of C130, FA12 and AC88.

19. The method of claim 17 wherein said antisense oligonucleotide comprises SEQ ID NO: 3.

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11/05/2002

Exhibit E

cells (page 4, first full paragraph). It is known in the art, however, that plural proteins can arise from a single gene through the processes of differential mRNA splicing or posttranslational modifications.

Claim 11 is clarified by amendment to recite "SEQ ID No. 1," as helpfully suggested by the Examiner.

Accordingly, Applicants respectfully request that the rejection of claims 10 and 11 under 35 U.S.C. §112, second paragraph, be withdrawn.

The 35 U.S.C. §112, first paragraph rejection

Claim 12 is rejected under 35 U.S.C §112, first paragraph, as not being enabled for the specific antibody C130; the specific hybridoma cell line required to practice claim 12 must be readily available to the public. This rejection is respectfully traversed.

A monoclonal antibody related to C130 is commercially available from Santa Cruz Biotechnology under the designation PACT (M56), as described in US Pat. No. 6,368,790 B1 (column 10, lines 50-52). Therefore, the required elements to practice claim 12 are

readily available to the public. Accordingly, Applicants respectfully request that the rejection of claim 12 under 35 U.S.C. §112, first paragraph, be withdrawn.

The 35 U.S.C. §102(b) rejection

Claims 10 and 11 are rejected under 35 U.S.C. §102(b) as being anticipated by either **Minoo et al. I** (Nov. 1989, *The Journal of Cell Biology*, Vol. 109, pages 1937-1946) or **Witte et al.** (1993, *Mol. Cell. Differ.* Vol. 2, pages 185-195). This rejection is respectfully traversed.

Claim 10 is amended to recite an isolated antibody that binds to a polypeptide encoded by an isolated DNA comprising SEQ ID No. 2, and that is prepared against said polypeptide. **Minoo** and **Witte** teach antibodies that bind to P2P protein, but which were prepared against proteins different from P2P. For example, antibody AC88 was prepared against the hsp90 protein; antibodies iD2 and fA12 were prepared against hnRNP core proteins (**Minoo**, page 1938). Such antibodies bind to P2P protein because P2P shares